

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (canceled).

2 (currently amended). The A method of claim 1, wherein the of assaying a *Brassica* plant for imidazolinone herbicide tolerance conferred by the PM1 mutation of the *B. napus AHAS1* gene, the method comprising the steps of:

- a) isolating genomic DNA from the plant;
- b) selectively amplifying an *AHAS1* gene from the genomic DNA using an *AHAS1* forward primer having a sequence as set forth in nucleotides 1 to 22 of SEQ ID NO:9 and an *AHAS1* reverse primer in a first amplification step, thereby producing an *AHAS1* reaction mixture;
- c) removing the *AHAS1* primers from the *AHAS1* reaction mixture to produce a purified *AHAS1* reaction mixture;
- d) in a second amplification step, further amplifying a portion of the amplified *AHAS1* gene containing the site of the PM1 mutation, by combining the purified *AHAS1* reaction mixture with a PM1 forward primer and a PM1 reverse primer, wherein the PM1 forward primer and the PM1 reverse primer bind to sites nested within the amplified portion of the *AHAS1* gene;
- e) denaturing the product of the second amplification step to produce single stranded polynucleotides that are allowed to adopt unique conformations by intramolecular interactions; and
- f) detecting the presence or absence of the PM1 mutation on the basis of the mobility of said single stranded polynucleotide conformers in a substrate.

3 (currently amended). The A method of claim 1, wherein of assaying a *Brassica* plant for imidazolinone herbicide tolerance conferred by the PM1 mutation of the *B. napus AHAS1* gene, the method comprising the steps of:

- a) isolating genomic DNA from the plant;
- b) selectively amplifying an *AHAS1* gene from the genomic DNA using an *AHAS1* forward primer and an *AHAS1* reverse primer has the having a sequence as set forth in nucleotides 1 to 22 of SEQ ID NO:10 in a first amplification step, thereby producing an *AHAS1* reaction mixture;
- c) removing the *AHAS1* primers from the *AHAS1* reaction mixture to produce a purified *AHAS1* reaction mixture;
- d) in a second amplification step, further amplifying a portion of the amplified *AHAS1* gene containing the site of the PM1 mutation, by combining the purified *AHAS1* reaction mixture with a PM1 forward primer and a PM1 reverse primer, wherein the PM1 forward primer and the PM1 reverse primer bind to sites nested within the amplified portion of the *AHAS1* gene;
- e) denaturing the product of the second amplification step to produce single stranded polynucleotides that are allowed to adopt unique conformations by intramolecular interactions; and
- f) detecting the presence or absence of the PM1 mutation on the basis of the mobility of said single stranded polynucleotide conformers in a substrate.

4 (currently amended). The method of claim 4 2 or 3, wherein the PM1 forward primer has a sequence as set forth in nucleotides 1 to 21 of SEQ ID NO:11.

5 (currently amended). The method of claim 4 2 or 3, wherein the PM1 reverse primer has a sequence as set forth in nucleotides 1 to 21 of SEQ ID NO:12.

6 (currently amended). The method of claim ~~4~~ or 3, wherein step e ~~(d)~~ includes incorporating a label into the amplified portion of the *AHAS1* gene.

7. The method of claim 6, wherein the label is selected from the group consisting of a radioactive label, a fluorescent label, a luminescent label, and a paramagnetic label.

8 (currently amended). The method of claim ~~4~~ or 3, wherein the substrate is selected from the group consisting of polyacrylamide, linear polyacrylamide, poly(N,N-dimethylacrylamide), hydroxylalkyl cellulose, polyoxyethylene, F127, agarose, diethylaminoethyl cellulose, sepharose, POP4, and POP6.

9 (currently amended). The method of claim ~~4~~ or 3, wherein the detection method is selected from the group consisting of electrophoresis and chromatography.

10 (currently amended). The method of claim ~~4~~ or 3, further comprising the step of detecting the presence or absence of PM2-mediated imidazolinone resistance in the plant.

11 (canceled).

12 (currently amended). The ~~A~~ method of claim 11, wherein ~~wherein the for assaying a Brassica plant for imidazolinone herbicide tolerance conferred by the PM2 mutation of the B. napus AHAS3 gene, the method comprising the steps of:~~

- a) isolating genomic DNA from the plant;
- b) selectively amplifying the AHAS3 gene from the genomic DNA using an AHAS3 forward primer having a sequence as set forth in nucleotides 1 to 22 of SEQ ID NO:13 and an AHAS3 reverse primer in a first amplification step to produce an AHAS3 reaction mixture;
- c) removing the AHAS3 primers from the AHAS3 reaction mixture to produce a purified AHAS3 reaction mixture;

- d) in a second amplification step, further amplifying the amplified AHAS3 gene, by combining a first aliquot of the purified AHAS3 reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for a wild type allele of the PM2 region at position 1712 of the AHAS3 gene as depicted in SEQ ID NOs:5 and 8;
- e) in a third amplification step further amplifying the amplified AHAS3 gene, by combining a second aliquot of the purified AHAS3 reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for the PM2 mutation; and
- f) analyzing the amplified first and second aliquots for the presence or absence of the PM2 mutation.

13 (currently amended). The A method of claim 11, wherein the for assaying a *Brassica* plant for imidazolinone herbicide tolerance conferred by the PM2 mutation of the *B. napus* AHAS3 gene, the method comprising the steps of:

- a) isolating genomic DNA from the plant;
- b) selectively amplifying the AHAS3 gene from the genomic DNA using an AHAS3 forward primer and an AHAS3 reverse primer having a sequence as set forth in nucleotides 1 to 23 of SEQ ID NO:14 in a first amplification step to produce an AHAS3 reaction mixture;
- c) removing the AHAS3 primers from the AHAS3 reaction mixture to produce a purified AHAS3 reaction mixture;
- d) in a second amplification step, further amplifying the amplified AHAS3 gene, by combining a first aliquot of the purified AHAS3 reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for a wild type allele of the PM2 region at position 1712 of the AHAS3 gene as depicted in SEQ ID NOs:5 and 8;

- e) in a third amplification step further amplifying the amplified AHAS3 gene, by combining a second aliquot of the purified AHAS3 reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for the PM2 mutation; and
- f) analyzing the amplified first and second aliquots for the presence or absence of the PM2 mutation.

14 (currently amended). The method of claim 44 12 or 13, wherein the PM2 region forward primer has a sequence as set forth in nucleotides 1 to 19 of SEQ ID NO:15.

15 (currently amended). The method of claim 44 12 or 13, wherein the PM2 region reverse primer has a sequence as set forth in nucleotides 1 to 19 of SEQ ID NO:16.

16 (currently amended). The method of claim 44 12 or 13, wherein the wild type allele of the PM2 region at position 1712 has a sequence as set forth in nucleotides 1 to 18 of SEQ ID NO:17.

17 (currently amended). The method of claim 44 12 or 13, wherein the primer selective for the PM2 mutation has a sequence as set forth in nucleotides 1 to 20 of SEQ ID NO:18.

18 (currently amended). The method of claim 44 12 or 13, wherein steps e (d) and d (e) include incorporating a label into the amplified portion of the AHAS3 gene.

19. The method of claim 18, wherein the label is selected from the group consisting of a radioactive label, a fluorescent label, a luminescent label, and a paramagnetic label.

20 (currently amended). The method of claim 44 12 or 13, wherein the analyzing step employs a method selected from the group consisting of electrophoresis and chromatography.

21 (currently amended). The method of claim 4+ 12 or 13, further comprising the steps of:

- g) selectively amplifying an *AHAS1* gene from the genomic DNA using an *AHAS1* forward primer and an *AHAS1* reverse primer in a fourth amplification step;
- h) removing the *AHAS1* primers from the product of step g);
- i) in a fifth amplification step, further amplifying a portion of the amplified *AHAS1* gene containing the site of the PM1 mutation, by combining the product of step h) with a PM1 forward primer and a PM1 reverse primer, wherein the PM1 forward primer and the PM1 reverse primer are bind to sites nested within the amplified portion of the *AHAS1* forward and reverse primers gene;
- j) denaturing the product of the fifth amplification step to produce single stranded polynucleotides that are allowed to adopt unique conformations by intramolecular interactions; and
- k) detecting the presence or absence of the PM1 mutation on the basis of the mobility of said single stranded conformer polynucleotides in a substrate.

22. (canceled).

23. (canceled).

24 (currently amended). A method of marker assisted breeding of plants of *Brassica* species using a PM1 mutation of the *B. napus AHAS1* gene as a marker, the method comprising the steps of:

- a) isolating genomic DNA from a *Brassica* plant;
- b) selectively amplifying an *AHAS1* gene from the genomic DNA using an *AHAS1* forward primer having a sequence as set forth in nucleotides 1

- to 22 of SEQ ID NO:9 and an *AHAS1* reverse primer in a first amplification step, thereby producing an *AHAS1* reaction mixture;
- c) removing the *AHAS1* primers from the *AHAS1* reaction mixture to produce a purified *AHAS1* reaction mixture;
 - d) in a second amplification step, further amplifying a portion of the amplified *AHAS1* gene containing the site of the PM1 mutation, by combining the purified *AHAS1* reaction mixture with a PM1 forward primer and a PM1 reverse primer, wherein the PM1 forward primer and the PM1 reverse primer are bind to sites nested within the amplified portion of the AHAS1 forward-and-reverse primers gene;
 - e) denaturing the product of the second amplification step to produce single stranded polynucleotides that are allowed to adopt unique conformations by intramolecular interactions;
 - f) detecting the presence or absence of the PM1 mutation on the basis of the mobility of said single stranded polynucleotide conformers in a substrate; and
 - g) selecting said plant as a parent for further breeding if the PM1 mutation is present.

25. A method of marker assisted breeding of plants of *Brassica* species using a PM2 mutation of the *B. napus AHAS3* gene as a marker, the method comprising the steps of:

- a) isolating genomic DNA from the plant;
- b) selectively amplifying the *AHAS3* gene from the genomic DNA using an *AHAS3* forward primer and an *AHAS3* reverse primer having a sequence as set forth in nucleotides 1 to 22 of SEQ ID NO:10 in a first amplification step to produce an *AHAS3* reaction mixture;
- c) removing the *AHAS3* primers from the *AHAS3* reaction mixture to produce a purified *AHAS3* reaction mixture;
- d) in a second amplification step, further amplifying the amplified *AHAS3* gene, by combining a first aliquot of the purified *AHAS3*

reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for a wild type allele of the PM2 region at position 1712 of the *AHAS3* gene as depicted in SEQ ID NOs:5 and 8;

- e) in a third amplification step further amplifying the amplified *AHAS3* gene, by combining a second aliquot of the purified *AHAS3* reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for the PM2 mutation;
- f) analyzing the amplified first and second aliquots for the presence or absence of the PM2 mutation; and
- g) selecting said plant as a parent for further breeding if the PM2 mutation is present.